THE RED STRING OF THE SUGAR-CANE

(*Bacillus pseudarabinus*, n.sp.).

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(Plates xiii.-xv.)

The presence of red-coloured vascular strings in the sugar-cane is not at all uncommon. The phenomenon appears to accompany several diseases of the cane, and at the same time canes which are to all appearance healthy may exhibit the colour. Among the diseases there are (1) the Sereh Disease, concerning the etiology of which there is much doubt; (2) the Sugar-Cane Disease of Massee, caused by *Trichosphaeria sacchari*, Massee,* and a similar disease, the Pine-apple Disease of the Cane, described by Went† as being produced by *Thielaviopsis ethaceticus*, Went; (3) the Red Smut of the Sugar-Cane, occasioned by *Colletotrichum falcatum*, Went, and this is undoubtedly identical with Cobb's Red Rot of the Sugar-Cane.‡ The instances of red string that I have investigated were not, however, related to any of these diseases, for they occurred in cases otherwise healthy and in plants affected with gummosis. The phenomenon must not be confused with the red-coloured tissue of some decayed canes, for in such cases the red colour only indicates that the cellular tissue is dead.

The first example of Red String that I examined was the case of an apparently healthy cane which had only three or four coloured bundles in cross section. The sample was small and

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* Massee, Ann. Bot. vii. 525; also in Text Book of Plant Diseases, London, 1899, 103, 365.

† Went, Ann. Bot. x. 583.

‡ Cobb, Agric. Gazette N.S. Wales, 1893.

contained many bacteria. A transverse section of one of the red bundles showed that the colour was caused by the presence of a red gum in the large vessels (Pl. xv., fig. 12). A red gum is also found in the Sereh Disease and in Red Smut, concerning both of which Went says that the gum is not caused by bacteria.

Other examples which I investigated were canes which were undoubtedly affected with gummosis; these were of the Chenoma variety which had been grown upon low-lying, poorly drained land.

Portions of the red strings were cut out with a sterile knife under aseptic conditions, and were inserted into tubes of molten ordinary glucose-gelatine, which after standing for an hour or two at 30° were poured into Petri-dishes. From the first and second samples I ultimately obtained a mould and several bacteria. In glucose-gelatine the mould produced a brilliant crimson-scarlet colour, and it undoubtedly was the agent which was primarily responsible for the colour of the strings. But from the presence of gum in the vessels I was of the opinion that the mould was accompanied by a slime bacterium, and that the complete phenomenon of red gum was brought about by the simultaneous growth of two organisms, a mould and a bacterium. This view was confirmed during the research. It may, however, be mentioned here that every portion of red vascular bundle that was taken did not contain the mould, but did contain slime-forming bacteria; and from this we must conclude that the mould does not accompany the gum along the whole length of the string, but colours the gum which is carried along the vessels, perhaps by sap-pressure, perhaps by bacterial growth, or that the rapid growth of bacteria starves out the mould after the colour has been produced. At any rate two things are certain, (1) the mould can under certain conditions produce the colour and cannot produce slime, and (2) the bacteria do produce slime.

In the original case three bacteria had been isolated. One of these I shall for convenience call the white slime bacterium, another was *Bact. sacchari*, the third was a race of *Bact. fluorescens liquefaciens*. To test which of these would produce a

crimson colour when grown in combination with the mould, the following experiment was made. A fragment of the mould was planted upon the centre of a plate of nutrient levulose-agar on which medium it seldom produces more than a trace of colour. When the mould had grown outwards as a zonate white pile of about 3 cm. diameter, the bacteria were infected at three places equidistant from the centre. In three days giant colonies had formed at the points of infection, while the mould had spread towards them. As the mould touched the white slime bacterial colony, a brilliant crimson colour developed not only throughout the colony but in the neighbouring medium. The colony of *Bact. sacchari* developed a foxy-red colour at the side towards the mould, and the medium was faintly stained the same colour. The mould refused to grow towards the colony of *Bact. fluorescens liquefaciens*, but grew around it, leaving a vacant space varying from 2 to 3 mm.

The experiment made it evident that of the three bacteria only one, viz., the white slime bacterium, could be of service to the mould in producing the colour of the crimson-red gum in the vessels of the cane. The nature of the slime, which was of the pasty consistency of cane-gum (the product of *Bact. vascularum*), also showed that it was well adapted for plugging up the large vessels of the vascular strings of the sugar-cane.

This bacterium grew upon fresh sterilised portions of sugar-cane as a white slime, while the mould during its growth upon the same substratum produced practically no colour,* the older cultures only showing spots of pinkish aerial hyphæ. But when both bacterium and mould were grown upon the cane, a deep crimson colour developed upon the outside of the cane where the

* Upon old portions of sugar-cane a red colour developed. Possibly the saccharose had become slowly inverted and the dextrose thus formed had induced the production of colour. Pigment appears to be determined by the presence of dextrose, other sugars or of gum. Saccharose and levulose generally do not cause a formation, but there appears to be no rule in the matter. Upon slices of potato sometimes the young growth is pink, sometimes it is white.

gum was forming. Upon cutting the cane across, many of the vascular strings were coloured; and finally the cotton wool upon which the cane rested also became crimson from the red gum which had flowed down the vessels of the strings. This experiment is, in my opinion, very significant as regards the combined (symbiotic) action of the mould and the bacterium in producing the red gum in the large vessels of the vascular strings of the sugar-cane.

I do not contend that this is the only bacterium that will assist in the red gum-formation, any more than I consider that the mould is the only fungus frequenting the cane that will produce a red colour; but it is clear from these experiments that the two are admirably adapted for producing the phenomenon. Although I have found the mould associated with *Bact. vasculorum* in a case of red string, yet these two did not produce the red colour upon sterile sugar-cane. Again, upon gelatine media the colour produced by these two is not so pronounced as with the mould and the white slime bacterium. The formation of a red colour upon sterile cane by the mould and *Bact. sacchari* was noted. A red gum traversed the vascular strings, but the colour was not so brilliant nor so much distributed as with the white slime bacterium.

The bacterium differed from those which I have previously obtained in forming white colonies and in producing a pasty slime upon levulose-asparagine-tannin agar. Several quantities of the slime were prepared by growing the bacterium upon this medium at different times, and from each quantity a solution of a gum was obtained by digestion in the autoclave. The gum mucilages prepared at different times gave identical reactions with certain reagents, so that these reactions may be taken as being constant and typical for the gum. The method of testing the gum is to place a drop of the thick mucilage upon a sheet of glass and to stir in a drop of the reagent. The following results were obtained. Basic and ammoniacal lead acetates gave a white clot, ferric chloride gave a brown clot, copper sulphate followed by potassium hydrate gave a blue precipitate which coagulated

but did not darken upon boiling the mixture in a test-tube. Sulphuric and phosphotungstic acids produced a curdy precipitate. No reactions were obtained with neutral lead acetate, barium hydrate, silver nitrate, mercuric chloride, copper sulphate, milk of lime, borax, iodine, Fehling's solution or Schweitzer's reagent.

With the exception of the phosphotungstic acid precipitate which is given with most bacterial gums, these reactions are identical with those given by arabin. But I did not, from the nature of the slime, expect the gum to be arabin, and this expectation was confirmed by the examination of the products of hydrolysis. After the gum had been freed from reducing sugars it was boiled for 5 hours with 5% sulphuric acid, when a portion was found to contain no gum precipitable by alcohol and to contain reducing substances. Fehling's solution was rather slowly reduced for a sugar, and as the whole portion gave but a trace of osazone it was evident that the gum was rather difficult to hydrolyse. In this respect it approached the pararabin gums. In two subsequent tests the gum was attacked by evaporating the solution in the water-bath with sulphuric acid until it began to char; then the solution was diluted with water to form a 10% solution which was boiled for two hours. A portion reduced Fehling's solution quickly, and upon the osazone being prepared and purified it was found to be galactosazone. Arabinosazone was carefully sought for at all stages of the purification, but it could not be detected. The gum was therefore a galactan, and was peculiar in giving the chemical reactions of arabin. In view of this behaviour, I propose to call the organism *Bacillus pseudarabinus*.

Bacillus pseudarabinus, n.sp.

Shape, etc.—The organism is a coccobacterium or short thick rod with rounded ends. In nutrient agar culture the rods measure 0·6 : 0·6-0·9μ, and in saccharose-potato-agar they are 0·7 : 0·8-1μ. In bouillon cultures the cells are actively motile. They stain readily, but are decolorised by the Gram method. The flagella are numerous and peritrichous; up to nine have been seen. No spores were observed.

Relation to oxygen.—The bacterium is a facultative anaërobe, there being a slight growth with gas-production under the mica plate in nutrient agar and saccharose-agar cultures.

Glucose-gelatine colonies.—They are raised, sometimes as hemispheres, circular, white and gummy. Microscopically they have granular radial striations from an almost opaque centre to the transparent edge. The deep colonies are opaque and rounded.

Nutrient gelatine colonies.—These are raised, white, glistening, and circular. Microscopically they are very finely granular with slightly erose margin and turbid or fibrous centre. The deep colonies are irregularly rounded, dark brown and somewhat zonate.

Nutrient agar colonies.—These are white, slightly raised, and from being round they become amœboid. Microscopically they are very finely granular. The deep colonies are oval or lenticular and semi-opaque.

Glucose-gelatine stab.—The needle track becomes filiform and rough. The nail-head is white, raised, and flat, with irregular margin. Gas bubbles are produced in the medium. By the 14th day the porcelain-white nail-head has become depressed centrally.

Glucose-gelatine stroke.—The growth is raised and white, with an irregular, erose margin. The colour becomes porcelain-white and the margin lobular. The growth becomes depressed from a slight liquefaction or rather consumption of the medium.

Nutrient gelatine stab.—More scanty, but otherwise similar to glucose-gelatine. A slight stratiform liquefaction had taken place by the 30th day.

Nutrient agar stroke.—A translucent white, broad, raised growth with spreading base is formed.

Bouillon.—The medium becomes very turbid, with a delicate white film and cohesive white sediment. The indol reaction was obtained and nitrates were reduced to nitrites.

Milk.—The medium had curdled by the 14th day and carried a slimy film.

Potato.—The growth is raised, fatty and dirty-white, but deepens to a flesh colour.

The Red Mould.

When infected upon the surface of nutrient glucose-gelatine the mould, when viewed from above, develops as a white, woolly growth. When seen from below, the surface of the medium is of a bright crimson-scarlet colour. The mycelium grows into the medium to about 5 mm. from the surface, while the colour slowly diffuses to lower depths. The gelatine is slowly liquefied. In faintly acid Hansen's glucose fluid* the mycelium grows throughout the liquid but soon covers the surface, producing a crimson velvety layer of hyphæ, the colour of which is communicated to the medium. Upon sterilised potato, rice, or sugar-cane, colour is not developed to any extent. The aerial hyphæ are white, while the ground hyphæ either remain white or become slightly olive-green. Then pale reddish prominences of hyphæ make their appearance, and as time goes on these are succeeded by black spots. Upon the surface of the potato or cane in contact with the glass wall of the test tube there is a progressive change of colour and variegated patches are produced.

Fragments of mycelium were sown in small hanging drops of nutrient fluid such as Hansen's fluid and ordinary nutrient bouillon. These sent out septate hyphæ, which branched and ramified in a horizontal plane. The refringent growing points of the hyphæ pierced the cell walls of neighbouring hyphæ which they chanced to meet, thus producing an anastomosing network. In some cases two parallel threads would present a step-ladder appearance. From the ground network, long delicate irregularly flattened hyphæ were sent into the air; these were sometimes studded with drops of fluid. In the damp chambers, these aerial hyphæ remained as such and never developed conidia or other organs of fructification; they simply existed as sharp-pointed aerial hyphæ. Besides these long and practically straight hyphæ there developed short, refringent, bent and twisted hyphæ.

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* Peptone 10, dextrose 50, potassium phosphate 3, magnesium sulphate 2, water 1000. Neutralise with potassium hydrate until 10 c.c. contains an acidity = 1 c.c. $^{N}/_{10}$ acid.

These rose at right angles to the plane of the glass and appeared to be of the nature of haustoria. Like the hyphæ of the ground mycelium, they were capable of penetrating other hyphæ, but, as they rose vertically, they did not have the same chances of doing so.

When the growth reached a maximum, some of the cells which constituted the anastomosing mycelium became thinner and less refractile, while other cells or groups of cells became stouter and the walls thicker. These thickened cells, however, were no more resistant to the entry of the growing point of a hypha than the ordinary cells, for the point could enter and abstract the contents not only of the cell which was entered but also of its neighbours, thus indicating that the individual thickened cells although divided by a septum were still in communication. These thickened cells were apparently of the nature of oidia.

In solutions of sugar such as Hansen's fluid no further development was observed, but in nutrient bouillon the thickened cells became gradually spherical, dark-coloured, and very granular. Sometimes single cells developed in this way, but generally several adjoining cells began to change (Pl. xiii., figs. 1-2). After becoming dark and opaque, many of the cells divided, usually at right angles to the direction of the original hypha, but not always so. Frequently these secondary cells again divided, and either became spherical or remained attached as hemispheres, forming a small moruloid cluster of cells. At this stage the growth ceased in the damp chambers.

It will be remembered that black patches appeared upon the surfaces of sterilised potato and sugar-cane which had been infected with the mould. Under a low magnification these appeared intensely black, dull or slightly shining, rough and moruloid, apparently consisting of a collection of irregular spheres (Pl. xiii., fig. 3). They were brittle, and under pressure broke up into opaque fragments, at the same time liberating a multitude of minute, colourless, egg-shaped spores, each measuring $5 : 3\mu$ (Pl. xiii., fig. 4). In order to gain an idea of the structure of these spore-cases or perithecia, paraffin sections of a potato culture

were made through a black area. The examination of these sections showed that the perithecia contained spores lying free in the cavity and surrounded by a single layer of black-coloured goblet cells, the apertures of which were directed into the cavity (Pl. xv., fig. 14). No other structures such as paraphyses or conidiophores were present. The section also showed smaller perithecia in the act of fusing, the dividing cellular walls being absorbed and the contents of the smaller perithecia joining to form a larger pycnidium (Pl. xv., fig. 15). The ready absorption of the cell walls indicates that the goblet cells were spherical or palisade cells, the inner ends of which had been dissolved by a cytase.

By this time it had become evident that the presence of sugar in the medium caused a proliferation of hyphæ, while the absence of soluble carbohydrates induced the formation of the spherical, dark-coloured cells. It also occurred to me that if small quantities of an almost sugarless but slightly nitrogenous medium were inserted into parts of a stiff starch-paste, we should have a condition of affairs more suited to the growth of the organism than could be obtained in hanging drops or on gelatine media. A trial showed that the method was valuable, inasmuch as it enabled phases of the development to be observed which were not visible by the usual methods. A 20 % starch-paste was prepared and poured into small Esmarch-dishes to a height of 1 cm. A drop of yeast-water, infected with spores from a perithecium, was introduced beneath the tough surface skin of the starch by means of a sterile capillary glass tube. In two days at 22° the surface of the starch at the point of infection was scantily covered with long, white, pinkish or greenish, branching septate aerial hyphæ, below which were many black points radiating in lines to the margin of the medium. These black points were the perithecia that were found in the potato and sugar-cane cultures. Imbedded in the starch-paste were numerous hyphæ which showed all conditions between the simple cell and the perithecium, thus enabling the various stages in the development of the perithecia to be observed (Pl. xv., fig. 13). When the cylindrical cell becomes

globular, it contains two globules which become four and then eight. The spherical cell then divides, and each portion or daughter-cell contains eight globules (Pl. xiii., fig. 6). These globules are the forerunners of the spores; they are devoid of a cell wall, for they coalesce to form one large globule upon applying pressure to the cover glass. Within the growing or dividing cell they are therefore naked, and only become covered when the process of division ceases. When this occurs the spherical aggregation of cells is a perithecium, the cells being the asci and the outer walls of the peripheral layer of cells being the covering. From the study of the paraffin potato sections, and of what occurs when an immature or a mature perithecium is crushed, it is clear that all the asci break down with the exception of those portions of the asci which constitute the covering of the perithecium. Thus the mould, during the process of forming the perithecium, passes through an ascigerous stage; in other words, during its development it is a member of the Ascomycetes probably akin to *Sphærella*, and when fully mature it is one of the Sphæropsideæ allied to *Phoma* but differing in the absence of conidiophores in the perithecium.

When the mould is sown upon the surface of sugar-nutrient gelatine in Petri-dishes there is formed first a white then a crimson growth of aerial hyphæ which may rise to a height of 3 mm. (Pl. xiv., fig. 8). Upon potato or sugar-cane, small patches of a rusty or pink colour sometimes appear in the white pile. When the growth is white, uncharacteristic hyphæ predominate, while in the red growths the terminal cells of the aerial hyphæ are generally swollen to a flask-shape (Pl. xiv., fig. 9). The contents of the swollen cells may be hyaline or granular. In size they vary considerably; on the surface of fluids such as Hansen's glucose media they are small, while on sugar-gelatine they are large. In many cases the penultimate cell can be seen growing into the flask-shaped terminal cell (Pl. xiv., fig. 11), and there is sometimes presented the appearance of the intergrowth becoming a cluster of cells within the cavity of the terminal cell. The intergrowth may emerge and continue to grow like an ordinary

hypha. The spherical cells behave as conidia or as any cell of the hyphæ, for when isolated and inserted in nutrient media they germinate in a normal fashion (Pl. xiv., fig. 10). The aerial hyphæ may produce the dark-coloured, thick-walled, spherical cells (asci), but this is exceptional. It was observed in the case of white hyphæ growing on sterile sugar-cane.

I cannot identify the mould with any hitherto described fungus, but that may be due to the fact that allied microscopic fungi are classified by means of the perithecia or pycnidia which are formed when the mould breaks through the epidermis of the host plant. This stage I have not seen. But as I have given other details, the mould will, I think, be recognised by future workers.

EXPLANATION OF PLATES XIII.-XV.

Plate xiii.

Figs. 1-2.—Development of the dark-coloured cells in hanging drop of ordinary nutrient bouillon (× 425).
Fig. 3.—Perithecia in outline on sterile sugar-cane (× 80).
Fig. 4.—Spores from a ruptured perithecium (× 425).
Fig. 5.—Spores germinating (× 425).
Fig. 6.—Chain of dark-coloured cells imbedded in starch-paste (× 500).
Fig. 7.—Cells from a ruptured immature perithecium (× 500).

Plate xiv.

Fig. 8.—Growth on the surface of levulose-asparagine-gelatine. Diagrammatic (× 2).
Fig. 9.—Flask-shaped terminal cells of aerial hyphæ (× 425).
Fig. 10.—Isolated terminal cell germinating (× 425).
Fig. 11.—Intergrowth by penultimate cells (× 425).

Plate xv.

Fig. 12.—Red gum in large vessel of vascular string. Section of fresh cane (× 75).
Fig. 13.—Formation of perithecia. Film from starch-paste (× 400).
Fig. 14.—Section of potato culture showing goblet cells of perithecial wall (× 400).
Fig. 15.—Section adjoining the same, showing absorption of wall and fusion of perithecia (× 400).